Liquid Triglycerides

IVD *In Vitro diagnostic* Kit

GPO-PAP Enzymatic Method

PO Code	Product Code	Pack Size	Kit Component
58075473	11033	5 x 20 mL	5 Bottles of R1 1 Bottle of Std

Intended Use

Reagent for the quantitative measurement of Triglycerides concentration in human serum or plasma samples.

Clinical Significance[1,2]

Triglycerides constitute 95% of tissue storage fat and their main role is to provide energy for the cell. They are synthesized both in the intestine from dietary carbohydrate and are then transported in blood by chylomicrons and VLDL. Serum Triglyceride values are found elevated in secondary hyperlipoproteinemia, atherosclerosis, glycogen storage diseases, nephrotic syndrome and greatly elevated in diabetes mellitus, chronic hepatitis and alcoholism.

Principle[1,2,3]

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by Adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2), proportional to the concentration of triglyceride in the sample.

Triglycerides + H_2O $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Fatty acid

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{GPO}}$ DHAP + H_2O_2

$2H_2O_2$ + Phenolic compound $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$ + 4-Aminoantipyrine

GPO: Glycerol 3 Phosphate oxidase
DHAP: Dihydroxyacetone Phosphate

The absorbance generated at 505 nm is proportional to the concentration of triglycerides in the sample.

Reagent Composition

Reagent 1
Pipes buffer (pH 7.00)
Mg2+
Chromogen
ATP
Potassium ferrocyanide
4-Aminoantipyrine
Lipoprotein Lipase
Glycerol Kinase
Glycerol-3-Phosphate Oxidase
Lipoprotein Lipase
Peroxidase
Stabilizers and Preservatives

Standard: Triglycerides 200 mg/dL

Waste Management

Disposal of all waste material should be in accordance with local and legal requirement.

Reagent Preparation, Storage and Stability

All reagents are ready to use liquid and stable up to the expiry date as stated on labels when stored at 2°C to 8°C.

Corrective Actions

The reagent should not be used
- When they do not exhibit the specified linearity or control values lie outside the acceptable range after recalibration
- When change in reagent color is observed
- After prolonged exposure to sunlight or high temperature

Sample collection, storage & stability[3,5]

Serum is preferred. EDTA or heparinized plasma can also be used.
Fasting (≥12 hours) samples are recommended for Triglycerides determination.
Samples should be used on the same day as far as possible. Samples are stable for 5 to 7 days when stored tightly capped at 2°C - 8°C.
Nevertheless, it is recommended to perform the assay with freshly collected samples.
Do not use hemolyzed or grossly contaminated samples.

General System Parameters#

Reaction Type	Endpoint
Reaction Slope	Increasing
Wavelength	505 nm
Reaction Temperature	37°C
Incubation	10 mins at 37° C
Sample Volume	10 µL
Reagent Volume	1000 µL
Pathlength	1 cm light path
Zero setting with	Reagent Blank

General System parameters can vary from instruments to instrument. Please use the above as a guidance.

Assay Procedure

Reagent and specimens must be at room temperature during preparation and testing.

	Blank	Standard	Test
Reagent 1	1000 µL	1000 µL	1000 µL
DI Water or Saline	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL
Mix well, incubate for 10 mins at 37° C and Read at 505nm			

Calculation of Results

$$\text{Trig Conc (mg/dL)} = \frac{\text{Abs of sample - Abs. of blank}}{\text{Abs of standard - Abs of blank}} \times \text{Conc. of Std*}$$

*Concentration of Standard as provided on the bottle.

Calibration

The assay can be calibrated with Standard provided with the kit.
Or A method specific Calibrator can be used to calibrate the assay.
We recommend AUTOPAK® Multi Calibrator (PO 58070877).
Or Any Standard Reference material (CRM/SRM) can be used to calibrate the assay.

Traceability

The assay is traceable to ID-GC-MS.

Quality control

To ensure adequate quality control, the use of control serum (Normal + Abnormal) is recommended with each assay batch. It is recommended that each laboratory establish its own acceptable range. It should be realized that the use of quality control material checks both, the instrument, and the reagent functions together. Follow government regulations or accreditation requirements for quality control frequency.

If the control values fall outside the specified limits, each of the below criteria should be cross-checked and corrected.

- Proper instrument function - wavelength setting, light source and
- temperature control
- Cleanliness of probes and cuvettes
- Bacterial contamination of wash water used by the instrument
- Expiry date of the reagent kit

Warning and Precautions

- Protect from a light and avoid contamination
- Do not freeze the reagent
- Do not interchange the components between different lots
- Do not interchange caps of reagent 1 & reagent 2

Material Required but Not Provided

Chemistry Analyzer or Equivalent, Micropipettes, Calibrator Quality control material.

Performance Characteristics

Linearity[4]

The assay is linear up to 1000 mg/dL, samples with value higher than 1000 mg/dL must be diluted 1:10 with normal saline and results multiplied by 10.

Sensitivity[4]

The lower limit of detection is 5 mg/dL Triglycerides.

Correlation[4]

A correlation was performed between AUTOPAK® reagent on AUTOPAK® 300 analyzer (Under Test) Vs reference predicate reagent.
The results were as follows:
Correlation coefficient: $r^2 = 0.9881$
Linear regression: y = 0.9626 x +9.8869

Precision[4]

		n	m	%CV
Within run Precision	**Level 1**	20	105	3.5
	Level 2	20	380	1.4

		n	m	%CV
Between run Precision	**Level 1**	50	107	2.1
	Level 2	50	386	1.7

Desirable Biological variation as per Westgard database 2014 for Triglycerides imprecision is 9.95% and Within subject Biologic variation is 19.9%. Source: https://www.westgard.com/biodatabase1.htm[5]

Interpretation of Results

Results obtained are under controlled conditions and may vary due to differences in laboratory environment, reagent and sample handling, instrument condition, etc. Laboratory is recommended to ensure that the differences observed in obtained results should not lead to an adverse impact on medical decision.

Interference Data[4]

Ascorbic Acid: No significant interference up to 2 mg/dL
Glucose: No significant interference up to 500 mg/dL
Bilirubin: No significant interference up to 25 mg/dL
Hemoglobin: No significant interference up to 250 mg/dL

Reference values[2]

It is recommended for each laboratory to establish its own Reference values.
The following values may be used as a guideline:

Normal	<150 mg/dL
Borderline High	150-199 mg/dL
Hightriglyceridemic	200-499 mg/dL
Very High	> 499 mg/dL

Bibliography

1. Tietz textbook of Clinical Chemistry, Third Edition. Carl A. Burtis and Edward R. Ashwood, eds. Philadelphia, PA: WB Saunders, 1998
2. Tietz Fundamentals of Clinical Chemistry, 6th edition. Carl A.Burtis, Edward R. Ashwood, and David E. Bruns,
3. Wu A, ed. Tietz Clinical Guide to Laboratory Tests, 4th ed. Philadelphia;
   W. B. Saunders Company, 2006
4. CLSI (NCCLS), Evaluation protocol EP09-A3, EP06-A, EP17-A, EP7-A2, EP15-A3 & EP05-A3.
5. www.westgard.com

Description of Symbols

IVD	*In vitro diagnostic* medical device		Manufacturer
REF	Product Code		Temperature Limit
LOT	Lot Number		Date of Manufacture
	Caution		Use by date
	Consult Instructions for use		

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